PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
C12N 15/82, 9/12, 15/54, A01H 5/00, 5/10	A1	**WO 98/10082**
		(43) International Publication Date: 12 March 1998 (12.03.98)

(21) International Application Number: PCT/US96/14244

(22) International Filing Date: 5 September 1996 (05.09.96)

(71) Applicant: UNIVERSITY OF FLORIDA [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).

(72) Inventors: GIROUX, Michael; S.W. 204 Kimball, Pullman, WA 99163 (US). HANNAH, L., Curtis; 4400 N.W. 39th Avenue #434, Gainesville, FL 32606 (US).

(74) Agents: SALIWANCHIK, David, R. et al.; Saliwanchik & Saliwanchik, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).

(81) Designated States: AL, AU, BB, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT

(57) Abstract

The subject invention pertains to novel variants of the maize gene, *Shrunken2(Sh2)* and a method of using that gene. The variant gene, *Sh2-m1Rev6*, encodes a subunit of the ADP-glucose pyrophosphorylase (AGP) enzyme that has additional amino acids inserted in or near the allosteric binding site of the protein. Corn seed expressing the *Sh2-m1Rev6* gene has a 15 % weight increase over wild type seed. The increase in seed weight is not associated simply with an increase in percentage starch content of the seed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

DESCRIPTION

MATERIALS AND METHODS FOR INCREASING CORN SEED WEIGHT

This invention was made with government support under National Science Foundation grant number 93052818. The government has certain rights in this invention.

Cross-Reference to a Related Application

This application is a continuation-in-part of co-pending application Serial No. 08/299,675, filed September 1, 1994.

Background of the Invention

ADP-glucose pyrophosphorylase (AGP) catalyzes the conversion of ATP and α-glucose-1-phosphate to ADP-glucose and pyrophosphate. ADP-glucose is used as a glycosyl donor in starch biosynthesis by plants and in glycogen biosynthesis by bacteria. The importance of ADP-glucose pyrophosphorylase as a key enzyme in the regulation of starch biosynthesis was noted in the study of starch deficient mutants of maize (*Zea mays*) endosperm (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). AGP enzymes have been isolated from both bacteria and plants. Bacterial AGP consists of a homotetramer, while plant AGP from photosynthetic and non-photosynthetic tissues is a heterotetramer composed of two different subunits. The plant enzyme is encoded by two different genes, with one subunit being larger than the other. This feature has been noted in a number of plants. The AGP subunits in spinach leaf have molecular weights of 54 kDa and 51 kDa, as estimated by SDS-PAGE. Both subunits are immunoreactive with antibody raised against purified AGP from spinach leaves (Copeland and Preiss, 1981; Morell *et al.*, 1987). Immunological analysis using antiserum prepared against the small and large subunits of spinach leaf showed that potato tuber AGP is also encoded by two genes (Okita *et al.*, 1990). The cDNA clones of the two subunits of potato tuber (50 and 51 kDa) have also been isolated and sequenced (Muller-Rober *et al.*, 1990; Nakata *et al.*, 1991).

As Hannah and Nelson (Hannah and Nelson, 1975 and 1976) postulated, both *Shrunken-2* (*Sh2*) (Bhave *et al.*, 1990) and *Brittle-2* (*Bt2*) (Bae *et al.*, 1990) are structural genes of maize endosperm ADP-glucose pyrophosphorylase. *Sh2* and *Bt2* encode the large subunit and small subunit of the enzyme, respectively. From cDNA sequencing, *Sh2* and *Bt2* proteins have predicted molecular weight of 57,179 Da (Shaw and Hannah, 1992) and 52,224 Da, respectively. The

endosperm is the site of most starch deposition during kernel development in maize. *Sh2* and *bt2* maize endosperm mutants have greatly reduced starch levels corresponding to deficient levels of AGP activity. Mutations of either gene have been shown to reduce AGP activity by about 95% (Tsai and Nelson, 1966; Dickinson and Preiss, 1969). Furthermore, it has been observed that enzymatic activities increase with the dosage of functional wild type *Sh2* and *Bt2* alleles, whereas mutant enzymes have altered kinetic properties. AGP is the rate limiting step in starch biosynthesis in plants. Stark *et al.* placed a mutant form of *E. coli* AGP in potato tuber and obtained a 35% increase in starch content (Stark, 1992).

The cloning and characterization of the genes encoding the AGP enzyme subunits have been reported for various plants. These include *Sh2* cDNA (Bhave *et al.*, 1990), *Sh2* genomic DNA (Shaw and Hannah, 1992), and *Bt2* cDNA (Bae *et al.*, 1990) from maize; small subunit cDNA (Anderson *et al.*, 1989) and genomic DNA (Anderson *et al.*, 1991) from rice; and small and large subunit cDNAs from spinach leaf (Morell *et al.*, 1987) and potato tuber (Muller-Rober *et al.*, 1990; Nakata *et al.*, 1991). In addition, cDNA clones have been isolated from wheat endosperm and leaf tissue (Olive *et al.*, 1989) and *Arabidopsis thaliana* leaf (Lin *et al.*, 1988).

AGP functions as an allosteric enzyme in all tissues and organisms investigated to date. The allosteric properties of AGP were first shown to be important in *E. coli*. A glycogen-overproducing *E. coli* mutant was isolated and the mutation mapped to the structural gene for AGP, designated as *glg*C. The mutant *E. coli*, known as *glg*C-16, was shown to be more sensitive to the activator, fructose 1,6 bisphosphate, and less sensitive to the inhibitor, cAMP (Preiss, 1984). Although plant AGP's are also allosteric, they respond to different effector molecules than bacterial AGP's. In plants, 3-phosphoglyceric acid (3-PGA) functions as an activator while phosphate (PO_4) serves as an inhibitor (Dickinson and Preiss, 1969).

In view of the fact that endosperm starch content comprises approximately 70% of the dry weight of the seed, alterations in starch biosynthesis correlate with seed weight. Unfortunately, the undesirable effect associated with such alterations has been an increase in the relative starch content of the seed. Therefore, the development of a method for increasing seed weight in plants without increasing the relative starch content of the seed is an object of the subject invention.

Brief Summary of the Invention

The subject invention concerns a novel variant of the *Shrunken-2* (*Sh2*) gene from maize. The *Sh2* gene encodes ADP-glucose pyrophosphorylase (AGP), an important enzyme involved in starch synthesis in the major part of the corn seed, the endosperm. In a preferred embodiment, the novel gene of the subject invention encodes a variant AGP protein which has two additional amino

acids inserted into the sequence. The variant gene described herein has been termed the *Sh2-m1Rev6* gene. Surprisingly, the presence of the *Sh2-m1Rev6* gene in a corn plant results in a substantial increase in corn seed weight when compared to wild type seed weight, but does so in the absence of an increase in the relative starch content of the kernel.

The subject invention further concerns a method of using the variant *sh2* gene in maize to increase seed weight. The subject invention also concerns plants having the variant *sh2* gene and expressing the mutant protein in the seed endosperm.

As described herein, the *sh*2 variant, *Sh2-m1Rev6*, can be produced using *in vivo*, site-specific mutagenesis. A transposable element was used to create a series of mutations in the sequence of the gene that encodes the enzyme. As a result, the *Sh2-m1Rev6* gene encodes an additional amino acid pair within or close to the allosteric binding site of the protein.

Brief Description of the Sequences

SEQ ID NO. 1 is the genomic nucleotide sequence of the *Sh2-m1Rev6* gene.

SEQ ID NO. 2 is the nucleotide sequence of the *Sh2-m1Rev6* cDNA.

SEQ ID NO. 3 is the amino acid sequence of the protein encoded by nucleotides 87 through 1640 of SEQ ID NO. 2.

SEQ ID NO. 4 is a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO. 5.

SEQ ID NO. 5 is the amino acid sequence of an ADP-glucose pyrophosphorylase (AGP) enzyme subunit containing a single serine insertion.

Detailed Disclosure of the Invention

The subject invention provides novel variants of the *Shrunken-2* (*Sh2*) gene and a method for increasing seed weight in a plant through the expression of the variant *sh2* gene. The *Sh2* gene encodes a subunit of the enzyme ADP-glucose pyrophosphorylase (AGP) in maize endosperm. One variant gene, denoted herein as *Sh2-m1Rev6*, contains an insertion mutation that encodes an additional tyrosine:serine or serine:tyrosine amino acid pair that is not present in the wild type protein. The sequences of the wild type DNA and protein are disclosed in Shaw and Hannah, 1992. The *in vivo*, site-specific mutation which resulted in the tyrosine:serine or serine:tyrosine insertion, was generated in *Sh2* using the transposable element, *dissociation* (*Ds*), which can insert into, and be excised from, the *Sh2* gene under appropriate conditions. *Ds* excision can alter gene expression through the addition of nucleotides to a gene at the site of excision of the element.

In a preferred embodiment, insertion mutations in the *Sh2* gene were obtained by screening for germinal revertants after excision of the *Ds* transposon from the gene. The revertants were generated by self-pollination of a stock containing the *Ds-Sh2* mutant allele, the *Activator* (*Ac*) element of this transposable element system, and appropriate outside markers. The *Ds* element can transpose when the *Ac* element is present. Wild type seed were selected, planted, self-pollinated and crossed onto a tester stock. Results from this test cross were used to remove wild type alleles due to pollen contamination. Seeds homozygous for each revertant allele were obtained from the self-progeny. Forty-four germinal revertants of the *Ds*-induced *sh2* mutant were collected.

Cloning and sequencing of the *Ds* insertion site showed that the nucleotide insertion resides in the area of the gene that encodes the binding site for the AGP activator, 3-PGA (Morrell, 1988). Of the 44 germinal revertants obtained, 28 were sequenced. The sequenced revertants defined 5 isoalleles of *sh2*: 13 restored the wild type sequence, 11 resulted in the insertion of the amino acid tyrosine, two contained an additional serine (inserted between amino acid residues 494 and 495, respectively, of the native protein sequence), one revertant contained a two amino acid insertion, tyrosine:tyrosine, and the last one, designated as *Sh2-m1Rev6*, contained the two amino acid insertion, tyrosine:serine or serine:tyrosine. The *Sh2-m1Rev6* variant encodes an AGP enzyme subunit that has either the serine:tyrosine amino acid pair inserted between the glycine and tyrosine at amino acid residues 494 and 495, respectively, of the native protein, or the serine:tyrosine amino acid pair inserted between the two tyrosine residues located at position 495 and 496 of the native protein sequence. Due to the sequence of the amino acids in the area of the insertions, the *Sh2-m1Rev6* variant amino acid sequences encoded by each of these insertions are identical to each other.

Surprisingly, the expression of the *Sh2-m1Rev6* gene in maize resulted in a significant increase in seed weight over that obtained from maize expressing the wild-type *Sh2* allele. Moreover, seeds from plants having the *Sh2-m1Rev6* gene contained approximately the same percentage starch content relative to any of the other revertants generated. In a preferred embodiment, the *Sh2-m1Rev6* gene is contained in homozygous form within the genome of a plant seed.

The subject invention further concerns a plant that has the *Sh2-m1Rev6* gene incorporated into its genome. Other alleles disclosed herein can also be incorporated into a plant genome. In a preferred embodiment, the plant is a monocotyledonous species. More preferably, the plant may be *Zea mays*. Plants having the *Sh2-m1Rev6* gene can be grown from seeds that have the gene in their genome. In addition, techniques for transforming plants with a gene are known in the art.

Because of the degeneracy of the genetic code, a variety of different polynucleotide sequences can encode the variant AGP polypeptide disclosed herein. In addition, it is well within

the skill of a person trained in the art to create alternative polynucleotide sequences encoding the same, or essentially the same, polypeptide of the subject invention. These variant or alternative polynucleotide sequences are within the scope of the subject invention. As used herein, references to "essentially the same" sequence refers to sequences which encode amino acid substitutions, deletions, additions, or insertions which do not materially alter the functional activity of the polypeptide encoded by *Sh2-m1Rev6* or the other alleles. The subject invention also contemplates those polynucleotide molecules having sequences which are sufficiently homologous with the wild type *Sh2* DNA sequence so as to permit hybridization with that sequence under standard high-stringency conditions. Such hybridization conditions are conventional in the art (see, *e.g.*, Maniatis *et al.*, 1989).

The polynucleotide molecules of the subject invention can be used to transform plants to express the *Sh2-m1Rev6* allele, or other alleles of the subject invention, in those plants. In addition, the polynucleotides of the subject invention can be used to express the recombinant variant AGP enzyme. They can also be used as a probe to detect related enzymes. The polynucleotides can also be used as DNA sizing standards.

The polypeptides encoded by the polynucleotides of the subject invention can be used to catalyze the conversion of ATP and α-glucose-1-phosphate to ADP-glucose and pyrophosphate, or to raise an immunogenic response to the AGP enzymes and variants thereof. They can also be used as molecular weight standards, or as an inert protein in an assay.

The following are examples which illustrate procedures and processes, including the best mode, for practicing the invention. These examples should not be construed as limiting, and are not intended to be a delineation of all possible modifications to the technique. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Expression of *Sh2-m1Rev6* Gene in Maize Endosperm.

Homozygous plants of each revertant obtained after excision of the *Ds* transposon were crossed onto the F1 hybrid corn, "Florida Stay Sweet." This sweet corn contains a null allele for the *Sh2* gene, termed *sh2*-R. Resulting endosperms contained one dose of the functional allele from a revertant and two female-derived null alleles, denoted by the following genotype *Sh2-m1RevX/sh2-R/sh2-R*, where X represents one of the various isoalleles of the revertants. Crosses were made during two growing seasons.

Resulting seed weight data for each revertant and wild type seed are shown in Table 1. The first column shows the amino acid insertion in the AGP enzyme obtained after the *in vivo*, site-specific mutagenesis.

Table 1.

Sequence alteration	# of revertants	Average Seed weight	Standard deviation
wild type	13	0.250 grams	0.015
tyrosine	11	0.238 grams	0.025
serine	2	0.261 grams	0.014
tyr, tyr	1	0.223 grams	nd*
tyr, ser (Rev6)	1	0.289 grams	0.022

**nd = not determined*

The data shown in Table 1 represents the average kernel seed weight for each revertant over the course of two growing seasons. The expression of the *Sh2-m1Rev6* gene to produce the Rev6 mutant AGP subunit gave rise to an almost 16% increase in seed weight in comparison to the wild type revertant. The revertants having the single serine insertion also showed an increase in average seed weight over wild type seed weight.

In addition, starch content was determined on the kernels analyzed above using various methodologies. The analysis showed that *Sh2-m1Rev6* containing kernels were no higher in percentage starch relative to kernels expressing the other alleles shown in the table above. Therefore, it appears that the increase in seed weight is not solely a function of starch content.

Corn seeds that contain at least one functional *Sh2-m1Rev6* allele (the tyrosine, serine insertion) have been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 USA, on May 20, 1996 and assigned ATCC accesion number ATCC 97624. Seeds having at least one functional *Sh2-m1Rev20* allele (serine insertion) have also been deposited with ATCC on May 20, 1996 and assigned ATCC accession number ATCC 97625.

The seeds have been deposited under conditions that assure that access to the biological material will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. The deposit will be available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood

that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject seed deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, *i.e.*, it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the seed. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject seed deposit will be irrevocably removed upon the granting of a patent disclosing it.

As would be apparent to a person of ordinary skill in the art, seeds and plants that are homozygous for the *Sh2-m1Rev6* or the *Sh2-m1Rev20* allele can be readily prepared from heterozygous seeds using techniques that are standard in the art. In addition, the *Sh2-m1Rev6* and *Sh2-m1Rev20* genes can be readily obtained from the deposited seeds.

The skilled artisan, using standard techniques known in the art, can also prepare polynucleotide molecules that encode additional amino acid residues, such as serine, at the location of the insertions in the subject revertants. Such polynucleotide molecules are included within the scope of the subject invention.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the scope and purview of this application and the scope of the appended claims.

References

Anderson, J.M., J. Hnilo, R. Larson, t.W. Okita, M. Morell, J. Preiss (1989) "The encoded primary sequence of a rice seed ADP-glucose pyrophosphorylase subunit and its homology to the bacterial enzyme," *J. Biol. Chem.* 264:12238-12242.

Anderson, J.M., R. Larson, D. Landencia, W.T. Kim, D. Morrow, T.W. Okita, J. Preiss (1991) "Molecular characterization of the gene encoding a rice endosperm-specific ADP-glucose pyrophosphorylase subunit and its developmental pattern of transcription," *Gene* 97:199-205.

Bae, J.M., M. Giroux, L.C. Hannah (1990) "Cloning and characterization of the *Brittle-2* gene of maize," *Maydica* 35:317-322.

Bhave, M.R., S. Lawrence, C. Barton, L.C. Hannah (1990) "Identification and molecular characterization of *Shrunken-2* cDNA clones of maize," *Plant Cell* 2:581-588.

Copeland, L., J. Preiss (1981) "Purification of spinach leaf ADP-glucose pyrophosphorylase," *Plant Physiol.* 68:996-1001.

Dickinson, D.B., J. Preiss (1969) "Presence of ADP-glucose pyrophosphorylase in *Shrunken-2* and *Brittle-2* mutants of maize endosperm," *Plant Physiol.* 44:1058-1062.

Hannah, L.C., O.E. Nelson (1975) "Characterization of adenosine diphosphate glucose pyrophosphorylase from developing maize seeds," *Plant Physiol.* 55:297-302.

Hannah, L.C., O.E. Nelson (1976) "Characterization of adenosine diphosphate glucose pyrophosphorylase from *Shrunken-2* and Brittle-2 mutants of maize," *Biochem. Genet.* 14:547-560.

Lin, T., T. Caspar, C. Somerville, J. Preiss (1988) "A starch deficient mutant of *Arabidopsis thaliana* with low ADP-glucose pyrophosphorylase activity lacks one of the two subunits of the enzyme," *Plant Physiol.* 88:1175-1181.

Maniatis, T., E.F. Fritsch, J. Sambrook (1989) *Molecular Cloning: A Laboratory Manual*, 2d Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Morell, M., M. Bloon, V. Knowles, J. Preiss (1988) "Subunit structure of spinach leaf ADP-glucose pyrophosphorylase," *J. Bio. Chem.* 263:633.

Muller-Rober, B.T., J. Kossmann, L.C. Hannah, L. Willmitzer, U. Sonnewald (1990) "One of the two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose," *Mol. Gen. Genet.* 224:136-146.

Nakata, P.A., T.W. Greene, J.M. Anderson, B.J. Smith-White, T.W. Okita, J. Preiss (1991) "Comparison of primary sequences of two potato tuber ADP-glucose pyrophosphorylase subunits," *Plant Mol. Biol.* 17:1089-1093.

Okita, T.W., P.A. Nakata, J.M. Anderson, J. Sowokinos, M. Morell, J. Preiss (1990) "The subunit structure of potato tuber ADP-glucose pyrophosphorylase," *Plant Physiol.* 93:785-790.

Olive, M.R., R.J. Ellis, W.W. Schuch (1989) "Isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase polypeptides from wheat leaf and endoosperm," *Plant Physiol. Mol. Biol.* 12:525-538.

Preiss, J. (1984) "Bacterial glycogen synthesis and it regulation," *Ann. Rev. Microbiol.* 419-458.

Shaw, J.R., L.C. Hannah (1992) "Genomic nucleotide sequence of a wild type *Shrunken-2* allele of *Zea mays*," *Plant Physiol.* 98:1214-1216.

Starke, *et al.* (1992) "Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase," *Science* 258:287.

Tsai, C., O.E. Nelson (1966) "Starch-deficient maize mutant lacking adenosine diphosphate glucose pyrophosphorylase activity," *Science* 151:341-343.

NOT FURNISHED UPON FILING

(A) LENGTH: 7745 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TAAGAGGGGT GCACCTAGCA TAGATTTTTT GGGCTCCCTG GCCTCTCCTT TCTTCCGCCT 60

GAAAACAACC TACATGGATA CATCTGCAAC CAGAGGGAGT ATCTGATGCT TTTTCCTGGG 120

CAGGGAGAGC TATGAGACGT ATGTCCTCAA AGCCACTTTG CATTGTGTGA AACCAATATC 180

GATCTTTGTT ACTTCATCAT GCATGAACAT TTGTGGAAAC TACTAGCTTA CAAGCATTAG 240

TGACAGCTCA GAAAAAAGTT ATCTCTGAAA GGTTTCATGT GTACCGTGGG AAATGAGAAA 300

TGTTGCCAAC TCAAACACCT TCAATATGTT GTTTGCAGGC AAACTCTTCT GGAAGAAAGG 360

TGTCTAAAAC TATGAACGGG TTACAGAAAG GTATAAACCA CGGCTGTGCA TTTTGGAAGT 420

ATCATCTATA GATGTCTGTT GAGGGGAAAG CCGTACGCCA ACGTTATTTA CTCAGAAACA 480

GCTTCAACAC ACAGTTGTCT GCTTTATGAT GGCATCTCCA CCCAGGCACC CACCATCACC 540

TATTCACCTA TCTCTCGTGC CTGTTTATTT TCTTGCCCTT TCTGATCATA AAAAATCATT 600

AAGAGTTTGC AAACATGCAT AGGCATATCA ATATGCTCAT TTATTAATTT GCTAGCAGAT 660

CATCTTCCTA CTCTTTACTT TATTTATTGT TTGAAAAATA TGTCCTGCAC CTAGGGAGCT 720

CGTATACAGT ACCAATGCAT CTTCATTAAA TGTGAATTTC AGAAAGGAAG TAGGAACCTA 780

TGAGAGTATT TTTCAAAATT AATTAGCGGC TTCTATTATG TTTATAGCAA AGGCCAAGGG 840

CAAAATCGGA ACACTAATGA TGGTTGGTTG CATGAGTCTG TCGATTACTT GCAAGAAATG 900

TGAACCTTTG TTTCTGTGCG TGGGCATAAA ACAAACAGCT TCTAGCCTCT TTTACGGTAC 960

TTGCACTTGC AAGAAATGTG AACTCCTTTT CATTTCTGTA TGTGGACATA ATGCCAAAGC 1020

ATCCAGGCTT TTTCATGGTT GTTGATGTCT TTACACAGTT CATCTCCACC AGTATGCCCT 1080

CCTCATACTC TATATAAACA CATCAACAGC ATCGCAATTA GCCACAAGAT CACTTCGGGA 1140

GGCAAGTGTG ATTTCGACCT TGCAGCCACC TTTTTTTGTT CTGTTGTAAG TATACTTTCC 1200

CTTACCATCT TTATCTGTTA GTTTAATTTG TAATTGGGAA GTATTAGTGG AAAGAGGATG 1260

AGATGCTATC ATCTATGTAC TCTGCAAATG CATCTGACGT TATATGGGCT GCTTCATATA 1320

ATTTGAATTG CTCCATTCTT GCCGACAATA TATTGCAAGG TATATGCCTA GTTCCATCAA 1380

AAGTTCTGTT TTTTCATTCT AAAAGCATTT TAGTGGCACG CAATTTTGTC CATGAGGGAA 1440

AGGAAATCTG TTTTGGTTAC TTTGCTTGAG GTGCATTCTT CATATGTCCA GTTTTATGGA 1500

AGTAATAAAC TTCAGTTTGG TCATAAGATG TCATATTAAA GGGCAAACAT ATATTCAATG 1560

TTCAATTCAT CGTAAATGTT CCCTTTTTGT AAAAGATTGC ATACTCATTT ATTTGAGTTG 1620

CAGGTGTATC TAGTAGTTGG AGGAGATATG CAGTTTGCAC TTGCATTGGA CACGAACTCA 1680

GGTCCTCACC AGATAAGATC TTGTGAGGGT GATGGGATTG ACAGGTTGGA AAAATTAAGT 1740

ATTGGGGGCA GAAAGCAGGA GAAAGCTTTG AGAAATAGGT GCTTTGGTGG TAGAGTTGCT 1800

GCAACTACAC AATGTATTCT TACCTCAGAT GCTTGTCCTG AAACTCTTGT AAGTATCCAC 1860

CTCAATTATT ACTCTTACAT GTTGGTTTAC TTTACGTTTG TCTTTTCAAG GGAAATTTAC 1920

TGTATTTTTT GTGTTTTGTG GGAGTTCTAT ACTTCTGTTG GACTGGTTAT TGTAAAGATT 1980

TGTTCAAATA GGGTCATCTA ATAATTGTTT GAAATCTGGG AACTGTGGTT TCACTGCGTT 2040

CAGGAAAAAG TGAATTATTG GTTACTGCAT GAATAACTTA TGGAAATAGA CCTTAGAGTT 2100

GCTGCATGAT TATCACAAAT CATTGCTACG ATATCTTATA ATAGTTCTTT CGACCTCGCA 2160

TTACATATAT AACTGCAACT CCTAGTTGCG TTCAAAAAAA AAAATGCAAC TCTTAGAACG 2220

CTCACCAGTG TAATCTTTCC TGAATTGTTA TTTAATGGCA TGTATGCACT ACTTGTATAC 2280

TTATCTAGGA TTAAGTAATC TAACTCTAGG CCCCATATTT GCAGCATTCT CAAACACAGT 2340

CCTCTAGGAA AAATTATGCT GATGCAAACC GTGTATCTGC TATCATTTTG GGCGGAGGCA 2400

CTGGATCTCA GCTCTTTCCT CTGACAAGCA CAAGAGCTAC GCCTGCTGTA AGGGATAACA 2460

CTGAACATCC AACGTTGATT ACTCTATTAT AGTATTATAC AGACTGTACT TTTCGAATTT 2520

ATCTTAGTTT TCTACAATAT TTAGTGGATT CTTCTCATTT TCAAGATACA CAATTGATCC 2580

ATAATCGAAG TGGTATGTAA GACAGTGAGT TAAAAGATTA TATTTTTTGG GAGACTTCCA 2640

GTCAAATTTT CTTAGAAGTT TTTTTGGTCC AGATGTTCAT AAAGTCGCCG CTTTCATACT 2700

TTTTTTAATT TTTTAATTGG TGCACTATTA GGTACCTGTT GGAGGATGTT ACAGGCTTAT 2760

TGATATCCCT ATGAGTAACT GCTTCAACAG TGGTATAAAT AAGATATTTG TGATGAGTCA 2820

GTTCAATTCT ACTTCGCTTA ACCGCCATAT TCATCGTACA TACCTTGAAG GCGGGATCAA 2880

CTTTGCTGAT GGATCTGTAC AGGTGATTTA CCTCATCTTG TTGATGTGTA ATACTGTAAT 2940

TAGGAGTAGA TTTGTGTGGA GAGAATAATA AACAGATGCC GAGATTCTTT TCTAAAAGTC 3000

TAGATCCAAA GGCATTGTGG TTCAAAACAC TATGGACTTC TACCATTTAT GTCATTACTT 3060

TGCCTTAATG TTCCATTGAA TGGGGCAAAT TATTGATTCT ACAAGTGTTT AATTAAAAAC 3120

TAATTGTTCA TCCTGCAGGT ATTAGCGGCT ACACAAATGC CTGAAGAGCC AGCTGGATGG 3180

TTCCAGGGTA CAGCAGACTC TATCAGAAAA TTTATCTGGG TACTCGAGGT AGTTGATATT 3240

TTCTCGTTTA TGAATGTCCA TTCACTCATT CCTGTAGCAT TGTTTCTTTG TAATTTTGAG 3300

TTCTCCTGTA TTTCTTTAGG ATTATTACAG TCACAAATCC ATTGACAACA TTGTAATCTT 3360

GAGTGGCGAT CAGCTTTATC GGATGAATTA CATGGAACTT GTGCAGGTAT GGTGTTCTCT 3420

TGTTCCTCAT GTTTCACGTA ATGTCCTGAT TTTGGATTAA CCAACTACTT TTGGCATGCA 3480

TTATTTCCAG AAACATGTCG AGGACGATGC TGATATCACT ATATCATGTG CTCCTGTTGA 3540

TGAGAGGTAA TCAGTTGTTT ATATCATCCT AATATGAATA TGTCATCTTG TTATCCAACA 3600

CAGGATGCAT ATGGTCTAAT CTGCTTTCCT TTTTTTTCCC TTCGGAAGCC GAGCTTCTAA 3660

AAATGGGCTA GTGAAGATTG ATCATACTGG ACGTGTACTT CAATTCTTTG AAAAACCAAA 3720

GGGTGCTGAT TTGAATTCTA TGGTTAGAAA TTCCTTGTGT AATCCAATTC TTTTGTTTTC 3780

CTTTCTTTCT TGAGATGAAC CCCTCTTTTA GTTATTTCCA TGGATAACCT GTACTTGACT 3840

TATTCAGAAA TGATTTTCTA TTTTGCTGTA GAATCTGACA CTAAAGCTAA TAGCACTGAT 3900

GTTGCAGAGA GTTGAGACCA ACTTCCTGAG CTATGCTATA GATGATGCAC AGAAATATCC 3960

ATACCTTGCA TCAATGGGCA TTTATGTCTT CAAGAAAGAT GCACTTTTAG ACCTTCTCAA 4020

GTAATCACTT TCCTGTGACT TATTTCTATC CAACTCCTAG TTTACCTTCT AACAGTGTCA 4080

ATTCTTAGGT CAAAATATAC TCAATTACAT GACTTTGGAT CTGAAATCCT CCCAAGAGCT 4140

GTACTAGATC ATAGTGTGCA GGTAAGTCTG ATCTGTCTGG AGTATGTGTT CTGTAAACTG 4200

TAAATTCTTC ATGTCAAAAA GTTGTTTTTG TTTCCAGTTT CCACTACCAA TGCACGATTT 4260

ATGTATTTTC GCTTCCATGC ATCATACATA CTAACAATAC ATTTTACGTA TTGTGTTAGG 4320

CATGCATTTT TACGGGCTAT TGGGAGGATG TTGGAACAAT CAAATCATTC TTTGATGCAA 4380

ACTTGGCCCT CACTGAGCAG GTACTCTGTC ATGTATTCTG TACTGCATAT ATATTACCTG 4440

GAATTCAATG CATAGAATGT GTTAGACCAT CTTAGTTCCA TCCTGTTTTC TTCAATTAGC 4500

TTATCATTTA ATAGTTGTTG GCTAGAATTT AAACACAAAT TTACCTAATA TGTTTCTCTC 4560

TTCAGCCTTC CAAGTTTGAT TTTTACGATC CAAAAACACC TTTCTTCACT GCACCCCGAT 4620

GCTTGCCTCC GACGCAATTG GACAAGTGCA AGGTATATGT CTTACTGAGC ACAATTGTTA 4680

CCTGAGCAAG ATTTTGTGTA CTTGACTTGT TCTCCTCCAC AGATGAAATA TGCATTTATC 4740

TCAGATGGTT GCTTACTGAG AGAATGCAAC ATCGAGCATT CTGTGATTGG AGTCTGCTCA 4800

CGTGTCAGCT CTGGATGTGA ACTCAAGGTA CATACTCTGC CAATGTATCT ACTCTTGAGT 4860

ATACCATTTC AACACCAAGC ATCACCAAAT CACACAGAAC AATAGCAACA AAGCCTTTTA 4920

GTTCCAAGCA ATTTAGGGTA GCCTAGAGTT GAAATCTAAC AAAACAAAAG TCAAAGCTCT 4980

ATCACGTGGA TAGTTGTTTT CCATGCACTC TTATTTAAGC TAATTTTTTG GGTATACTAC 5040

ATCCATTTAA TTATTGTTTT ATTGCTTCTT CCCTTTGCCT TTCCCCCATT ACTATCGCGT 5100

CTTAAGATCA TACTACGCAC TAGTGTCTTT AGAGGTCTCT GGTGGACATG TTCAAACCAT 5160

CTCAATCGGT GTTGGACAAG TTTTTCTTGA ATTTGTGCTA CACCTAACCT ATCACGTATG 5220

TCATCGTTTC AAACTCGATC CTTCCTGTAT CATCATAAAT CCAATGCAAC ATACGCATTT 5280

ATGCAACATT TATCTGTTGA ACATGTCATC TTTTTGTAGG TTAACATTAT GCACCATACA 5340

ATGTAGCATG TCTAATCATC ATCCTATAAA ATTTACATTT TAGCTTATGT GGTATCCTCT 5400

TGCCACTTAG AACACCATAT GCTTGATGCC ATTTCATCCA CCCTGCTTTG ATTCTATGGC 5460

TAACATCTTC ATTAATATCC TCGCCTCTCT GTATCATTGG TCCTAAATAT GGAAATACAT 5520

TCTTTCTGGG CACTACTTGA CCTTCCAAAC TAACGTCTCC TTTGCTCCTT TCTTGTGTGT 5580

AGTAGTACCG AAGTCACATC TCATATATTC GGTTTTAGTT CTACTAAGTC CCGGGTTCGA 5640

TCCCCCTCAG GGGTGAATTT CGGGCTTGGT AAAAAAAATC CCCTCGCTGT GTCCCGCCCG 5700

CTCTCGGGGA TCGATATCCT GCGCGCCACC CTCCGGCTGG GCATTGCAGA GTGAGCAGTT 5760

GATCGGCTCG TTAGTGATGG GGAGCGGGGT TCAAGGGTTT TCTCGGCCGG GACCATGTTT 5820

CGGTCTCTTA ATATAATGCC GGGAGGGCAG TCTTTCCCTC CCCGGTCGAG TTTTAGTTCT 5880

ACCGAGTCTA AAACCTTTGG ACTCTAGAGT CCCCTGTCAC AACTCACAAC TCTAGTTTTC 5940

TATTTACTTC TACCTAGCGT TTATTAATGA TCACTATATC GTCTGTAAAA AGCATACACC 6000

AATGTAATCC CCTTGTATGT CCCTTGTAAT ATTATCCATC ACAAGAAAAA AAGGTAAGGC 6060

TCAAAGTTGA CTTTTGATAT AGTCCTATTC TAATCGAGAA GTCATCTGTA TCTTCGTCTC 6120

TTGTTCGAAC ACTAGTCACA AAATTTTTTG TACATGTTCT TAATGAGTCC AACGTAATAT 6180

TCCTTGATAT TTTGTCATAA GCCCTCATCA AGTCAATGAA AATCACGTGT AGGTCCTTCA 6240

TTTGTTCCTT ATACTGCTCC ATCACTTGTC TCATTAAGAA AATCTCTCTC ATAGTTAACC 6300

TTTTGGCATG AAACAAAATC ACACAGAAGT TGTTTCCTTT TTTTAAGATC CCACACAAAA 6360

GAGGTTTGAT CTAAGGAATC TGGATCCCTG ACAGGTTTAT CAAAATCCTT TGTGTTTTTC 6420

TTAAAACTGA ATATTCCTCC AGCTTCTAGT ATTGATGTAA TATTCAATCT GTTTAGCAAG 6480

TGAACACCTT GGTTCTTGTT GTTACTGTAC CCCCCCCCCC CCCCCCCCCC CGAGGCCCAG 6540

ATTACCACGA CATGAATACA AGAATATTGA ACCCAGATCT AGAGTTTGTT TGTACTGTTG 6600

AAAATCGGTG ACAATTCATT TTGTTATTGC GCTTTCTGAT AACGACAGGA CTCCGTGATG 6660

ATGGGAGCGG ACACCTATGA AACTGAAGAA GAAGCTTCAA AGCTACTGTT AGCTGGGAAG 6720

GTCCCAGTTG GAATAGGAAG GAACACAAAG ATAAGGTGAG TATGGATGTG GAACCACCGG 6780

TTAGTTCCCA AAAATATCAC TCACTGATAC CTGATGGTAT CCTCTGATTA TTTTCAGGAA 6840

CTGTATCATT GACATGAATG CTAGGATTGG GAAGAACGTG GTGATCACAA ACAGTAAGGT 6900

GAGCGAGCGC ACCTACATGG GTGCAGAATC TTGTGTGCTC ATCTATCCTA ATTCGGTAAT 6960

TCCTATCCAG CGCTAGTCTT GTGACCATGG GGCATGGGTT CGACTCTGTG ACAGGGCATC 7020

CAAGAGGCTG ATCACCCGGA AGAAGGGTAC TCGTACTACA TAAGGTCTGG AATCGTGGTG 7080

ATCTTGAAGA ATGCAACCAT CAACGATGGG TCTGTCATAT AGATCGGCTG CGTGTGCGTC 7140

TACAAAACAA GAACCTACAA TGGTATTGCA TCGATGGATC GTGTAACCTT GGTATGGTAA 7200

GAGCCGCTTG ACAGAAAGTC GAGCGTTCGG GCAAGATGCG TAGTCTGGCA TGCTGTTCCT 7260

TGACCATTTG TGCTGCTAGT ATGTACTGTT ATAAGCTGCC CTAGAAGTTG CAGCAAACCT 7320

TTTTATGAAC CTTTGTATTT CCATTACCTG CTTTGGATCA ACTATATCTG TCATCCTATA 7380

TATTACTAAA TTTTTACGTG TTTTTCTAAT TCGGTGCTGC TTTTGGGATC TGGCTTCGAT 7440

GACCGCTCGA CCCTGGGCCA TTGGTTCAGC TCTGTTCCTT AGAGCAACTC CAAGGAGTCC 7500

TAAATTTTGT ATTAGATACG AAGGACTTCA GCCGTGTATG TCGTCCTCAC CAAACGCTCT 7560

TTTTGCATAG TGCAGGGGTT GTAGACTTGT AGCCCTTGTT TAAAGAGGAA TTTGAATATC 7620

AAATTATAAG TATTAAATAT ATATTTAATT AGGTTAACAA ATTTGGCTCG TTTTTAGTCT 7680

TTATTTATGT AATTAGTTTT AAAAATAGAC CTATATTTCA ATACGAAATA TCATTAACAT 7740

CGATA 7745

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1919 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACAAGATCAC TTCGGGAGGC AAGTGCGATT TTGATCTTGC AGCCACCTTT TTTTGTTCTG 60

TTGTGTATCT AGTAGTTGGA GGAGATATGC AGTTTGCACT TGCATTGGAC ACGAACTCAG 120

GTCCTCACCA GATAAGATCT TGTGAGGGTG ATGGGATTGA CAGGTTGGAA AAATTAAGTA 180

TTGGGGGCAG AAAGCAGGAG AAAGCTTTGA GAAATAGGTG CTTTGGTGGT AGAGTTGCTG 240

CAACTACACA ATGTATTCTT ACCTCAGATG CTTGTCCTGA AACTCTTCAT TCTCAAACAC 300

AGTCCTCTAG GAAAAATTAT GCTGATGCAA ACCGTGTATC TGCGATCATT TTGGGCGGAG 360

GCACTGGATC TCAGCTCTTT CCTCTGACAA GCACAAGAGC TACGCCTGCT GTACCTGTTG 420

GAGGATGTTA CAGGCTTATT GATATCCCTA TGAGTAACTG CTTCAACAGT GGTATAAATA 480

AGATATTTGT GATGAGTCAG TTCAATTCTA CTTCGCTTAA CCGCCATATT CATCGTACAT 540

ACCTTGAAGG CGGGATCAAC TTTGCTGATG GATCTGTACA GGTATTAGCG GCTACACAAA 600

TGCCTGAAGA GCCAGCTGGA TGGTTCCAGG GTACAGCAGA CTCTATCAGA AAATTTATCT 660

GGGTACTCGA GGATTATTAC AGTCACAAAT CCATTGACAA CATTGTAATC TTGAGTGGCG 720

ATCAGCTTTA TCGGATGAAT TACATGGAAC TTGTGCAGAA ACATGTCGAG GACGATGCTG 780

ATATCACTAT ATCATGTGCT CCTGTTGATG AGAGCCGAGC TTCTAAAAAT GGGCTAGTGA 840

AGATTGATCA TACTGGACGT GTACTTCAAT TCTTTGAAAA ACCAAAGGGT GCTGATTTGA 900

ATTCTATGAG AGTTGAGACC AACTTCCTGA GCTATGCTAT AGATGATGCA CAGAAATATC 960

CATACCTTGC ATCAATGGGC ATTTATGTCT TCAAGAAAGA TGCACTTTTA GACCTTCTCA 1020

AGTCAAAATA TACTCAATTA CATGACTTTG GATCTGAAAT CCTCCCAAGA GCTGTACTAG 1080

ATCATAGTGT GCAGGCATGC ATTTTTACGG GCTATTGGGA GGATGTTGGA ACAATCAAAT 1140

CATTCTTTGA TGCAAACTTG GCCCTCACTG AGCAGCCTTC CAAGTTTGAT TTTTACGATC 1200

CAAAAACACC TTTCTTCACT GCACCCCGAT GCTTGCCTCC GACGCAATTG GACAAGTGCA 1260

AGATGAAATA TGCATTTATC TCAGATGGTT GCTTACTGAG AGAATGCAAC ATCGAGCATT 1320

CTGTGATTGG AGTCTGCTCA CGTGTCAGCT CTGGATGTGA ACTCAAGGAC TCCGTGATGA 1380

TGGGAGCGGA CATCTATGAA ACTGAAGAAG AAGCTTCAAA GCTACTGTTA GCTGGGAAGG 1440

TCCCGATTGG AATAGGAAGG AACACAAAGA TAAGGAACTG TATCATTGAC ATGAATGCTA 1500

GGATTGGGAA GAACGTGGTG ATCACAAACA GTAAGGGCAT CCAAGAGGCT GATCACCCGG 1560

AAGAAGGGTA CTCGTACTAC ATAAGGTCTG GAATCGTGGT GATCCTGAAG AATGCAACCA 1620

TCAACGATGG GTCTGTCATA TAGATCGGCT GCGTTTGCGT CTACAAAACA AGAACCTACA 1680

ATGGTATTGC ATCGATGGAT CGTGTAACCT TGGTATGGTA AGAGCCGCTT GACAGGAAGT 1740

CGAGCTTCGG GCGAAGATGC TAGTCTGGCA TGCTGTTCCT TGACCATTTG TGCTGCTAGT 1800

ATGTACCTGT TATAAGCTGC CCTAGAAGTT GCAGCAAACC TTTTTATGAA CCTTTGTATT 1860

TCCATTACCC TGCTTTGGAT CAACTATATC TGTCAGTCCT ATATATTACT AAATTTTTA 1919

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gln Phe Ala Leu Ala Leu Asp Thr Asn Ser Gly Pro His Gln Ile
1 5 10 15

Arg Ser Cys Glu Gly Asp Gly Ile Asp Arg Leu Glu Lys Leu Ser Ile
20 25 30

Gly Gly Arg Lys Gln Glu Lys Ala Leu Arg Asn Arg Cys Phe Gly Gly
35 40 45

Arg Val Ala Ala Thr Thr Gln Cys Ile Leu Thr Ser Asp Ala Cys Pro
50 55 60

Glu Thr Leu His Ser Gln Thr Gln Ser Ser Arg Lys Asn Tyr Ala Asp
65 70 75 80

Ala Asn Arg Val Ser Ala Ile Ile Leu Gly Gly Gly Thr Gly Ser Gln
85 90 95

Leu Phe Pro Leu Thr Ser Thr Arg Ala Thr Pro Ala Val Pro Val Gly
100 105 110

Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Phe Asn Ser
115 120 125

Gly Ile Asn Lys Ile Phe Val Met Ser Gln Phe Asn Ser Thr Ser Leu
130 135 140

Asn Arg His Ile His Arg Thr Tyr Leu Glu Gly Gly Ile Asn Phe Ala
145 150 155 160

Asp Gly Ser Val Gln Val Leu Ala Ala Thr Gln Met Pro Glu Glu Pro
165 170 175

Ala Gly Trp Phe Gln Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp
180 185 190

Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile
195 200 205

Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln
210 215 220

Lys His Val Glu Asp Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val
225 230 235 240

Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr
245 250 255

Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn
260 265 270

Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala
275 280 285

Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys
290 295 300

Asp Ala Leu Leu Asp Leu Leu Lys Ser Lys Tyr Thr Gln Leu His Asp
305 310 315 320

Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln
325 330 335

Ala Cys Ile Phe Thr Gly Tyr Trp Glu Asp Val Gly Thr Ile Lys Ser
340 345 350

Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp
355 360 365

Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro
370 375 380

Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp
385 390 395 400

Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val
405 410 415

Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met
420 425 430

Gly Ala Asp Ile Tyr Glu Thr Glu Glu Glu Ala Ser Lys Leu Leu Leu
435 440 445

Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn
450 455 460

Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr
465 470 475 480

Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Tyr Ser
485 490 495

Tyr Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile
500 505 510

Asn Asp Gly Ser Val Ile
515

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1551 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATGCAGTTTG CACTTGCATT GGACACGAAC TCAGGTCCTC ACCAGATAAG ATCTTGTGAG 60

GGTGATGGGA TTGACAGGTT GGAAAAATTA AGTATTGGGG GCAGAAAGCA GGAGAAAGCT 120

TTGAGAAATA GGTGCTTTGG TGGTAGAGTT GCTGCAACTA CACAATGTAT TCTTACCTCA 180

GATGCTTGTC CTGAAACTCT TCATTCTCAA ACACAGTCCT CTAGGAAAAA TTATGCTGAT 240

GCAAACCGTG TATCTGCGAT CATTTTGGGC GGAGGCACTG GATCTCAGCT CTTTCCTCTG 300

ACAAGCACAA GAGCTACGCC TGCTGTACCT GTTGGAGGAT GTTACAGGCT TATTGATATC 360

CCTATGAGTA ACTGCTTCAA CAGTGGTATA AATAAGATAT TTGTGATGAG TCAGTTCAAT 420

TCTACTTCGC TTAACCGCCA TATTCATCGT ACATACCTTG AAGGCGGGAT CAACTTTGCT 480

GATGGATCTG TACAGGTATT AGCGGCTACA CAAATGCCTG AAGAGCCAGC TGGATGGTTC 540

CAGGGTACAG CAGACTCTAT CAGAAAATTT ATCTGGGTAC TCGAGGATTA TTACAGTCAC 600

AAATCCATTG ACAACATTGT AATCTTGAGT GGCGATCAGC TTTATCGGAT GAATTACATG 660

GAACTTGTGC AGAAACATGT CGAGGACGAT GCTGATATCA CTATATCATG TGCTCCTGTT 720

GATGAGAGCC GAGCTTCTAA AAATGGGCTA GTGAAGATTG ATCATACTGG ACGTGTACTT 780

CAATTCTTTG AAAAACCAAA GGGTGCTGAT TTGAATTCTA TGAGAGTTGA GACCAACTTC 840

CTGAGCTATG CTATAGATGA TGCACAGAAA TATCCATACC TTGCATCAAT GGGCATTTAT 900

GTCTTCAAGA AAGATGCACT TTTAGACCTT CTCAAGTCAA AATATACTCA ATTACATGAC 960

TTTGGATCTG AAATCCTCCC AAGAGCTGTA CTAGATCATA GTGTGCAGGC ATGCATTTTT 1020

ACGGGCTATT GGGAGGATGT TGGAACAATC AAATCATTCT TTGATGCAAA CTTGGCCCTC 1080

ACTGAGCAGC CTTCCAAGTT TGATTTTTAC GATCCAAAAA CACCTTTCTT CACTGCACCC 1140

CGATGCTTGC CTCCGACGCA ATTGGACAAG TGCAAGATGA AATATGCATT TATCTCAGAT 1200

GGTTGCTTAC TGAGAGAATG CAACATCGAG CATTCTGTGA TTGGAGTCTG CTCACGTGTC 1260

AGCTCTGGAT GTGAACTCAA GGACTCCGTG ATGATGGGAG CGGACATCTA TGAAACTGAA 1320

GAAGAAGCTT CAAAGCTACT GTTAGCTGGG AAGGTCCCGA TTGGAATAGG AAGGAACACA 1380

AAGATAAGGA ACTGTATCAT TGACATGAAT GCTAGGATTG GGAAGAACGT GGTGATCACA 1440

AACAGTAAGG GCATCCAAGA GGCTGATCAC CCGGAAGAAG GGTCCTACTA CATAAGGTCT 1500

GGAATCGTGG TGATCCTGAA GAATGCAACC ATCAACGATG GGTCTGTCAT A 1551

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gln Phe Ala Leu Ala Leu Asp Thr Asn Ser Gly Pro His Gln Ile
1 5 10 15

Arg Ser Cys Glu Gly Asp Gly Ile Asp Arg Leu Glu Lys Leu Ser Ile
20 25 30

Gly Gly Arg Lys Gln Glu Lys Ala Leu Arg Asn Arg Cys Phe Gly Gly
35 40 45

Arg Val Ala Ala Thr Thr Gln Cys Ile Leu Thr Ser Asp Ala Cys Pro
50 55 60

Glu Thr Leu His Ser Gln Thr Gln Ser Ser Arg Lys Asn Tyr Ala Asp
65 70 75 80

Ala Asn Arg Val Ser Ala Ile Ile Leu Gly Gly Gly Thr Gly Ser Gln
85 90 95

Leu Phe Pro Leu Thr Ser Thr Arg Ala Thr Pro Ala Val Pro Val Gly
100 105 110

Gly Cys Tyr Arg Leu Ile Asp Ile Pro Met Ser Asn Cys Phe Asn Ser
115 120 125

Gly Ile Asn Lys Ile Phe Val Met Ser Gln Phe Asn Ser Thr Ser Leu
130 135 140

```
Asn Arg His Ile His Arg Thr Tyr Leu Glu Gly Gly Ile Asn Phe Ala
145                 150                 155                 160

Asp Gly Ser Val Gln Val Leu Ala Ala Thr Gln Met Pro Glu Glu Pro
                165                 170                 175

Ala Gly Trp Phe Gln Gly Thr Ala Asp Ser Ile Arg Lys Phe Ile Trp
            180                 185                 190

Val Leu Glu Asp Tyr Tyr Ser His Lys Ser Ile Asp Asn Ile Val Ile
        195                 200                 205

Leu Ser Gly Asp Gln Leu Tyr Arg Met Asn Tyr Met Glu Leu Val Gln
    210                 215                 220

Lys His Val Glu Asp Asp Ala Asp Ile Thr Ile Ser Cys Ala Pro Val
225                 230                 235                 240

Asp Glu Ser Arg Ala Ser Lys Asn Gly Leu Val Lys Ile Asp His Thr
                245                 250                 255

Gly Arg Val Leu Gln Phe Phe Glu Lys Pro Lys Gly Ala Asp Leu Asn
            260                 265                 270

Ser Met Arg Val Glu Thr Asn Phe Leu Ser Tyr Ala Ile Asp Asp Ala
        275                 280                 285

Gln Lys Tyr Pro Tyr Leu Ala Ser Met Gly Ile Tyr Val Phe Lys Lys
    290                 295                 300

Asp Ala Leu Leu Asp Leu Leu Lys Ser Lys Tyr Thr Gln Leu His Asp
305                 310                 315                 320

Phe Gly Ser Glu Ile Leu Pro Arg Ala Val Leu Asp His Ser Val Gln
                325                 330                 335

Ala Cys Ile Phe Thr Gly Tyr Trp Glu Asp Val Gly Thr Ile Lys Ser
            340                 345                 350

Phe Phe Asp Ala Asn Leu Ala Leu Thr Glu Gln Pro Ser Lys Phe Asp
        355                 360                 365

Phe Tyr Asp Pro Lys Thr Pro Phe Phe Thr Ala Pro Arg Cys Leu Pro
    370                 375                 380

Pro Thr Gln Leu Asp Lys Cys Lys Met Lys Tyr Ala Phe Ile Ser Asp
385                 390                 395                 400

Gly Cys Leu Leu Arg Glu Cys Asn Ile Glu His Ser Val Ile Gly Val
                405                 410                 415

Cys Ser Arg Val Ser Ser Gly Cys Glu Leu Lys Asp Ser Val Met Met
            420                 425                 430

Gly Ala Asp Ile Tyr Glu Thr Glu Glu Glu Ala Ser Lys Leu Leu Leu
        435                 440                 445
```

Ala Gly Lys Val Pro Ile Gly Ile Gly Arg Asn Thr Lys Ile Arg Asn
450 455 460

Cys Ile Ile Asp Met Asn Ala Arg Ile Gly Lys Asn Val Val Ile Thr
465 470 475 480

Asn Ser Lys Gly Ile Gln Glu Ala Asp His Pro Glu Glu Gly Ser Tyr
485 490 495

Tyr Ile Arg Ser Gly Ile Val Val Ile Leu Lys Asn Ala Thr Ile Asn
500 505 510

Asp Gly Ser Val Ile
515

Claims

1. A polynucleotide molecule, comprising a variant of the wild type *shrunken-2* (*Sh2*) gene, wherein said variant codes for the insertion of at least one additional amino acid within or close to the allosteric binding site of the ADP-glucose pyrophosphorylase (AGP) enzyme subunit, whereby a plant expressing said polynucleotide molecule has increased seed weight relative to the seed weight of a plant expressing the wild type *Sh2* gene.

2. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes at least one serine residue inserted between amino acids 494 and 495 of the native AGP enzyme subunit.

3. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes the amino acid pair tyrosine:serine, wherein said amino acid pair is inserted between amino acids 494 and 495 of the native AGP enzyme subunit.

4. The polynucleotide molecule, according to claim 1, wherein said polynucleotide molecule encodes the amino acid pair serine:tyrosine, wherein said amino acid pair is inserted between amino acids 495 and 496 of the native AGP enzyme subunit.

5. The polynucleotide molecule, according to claim 1, wherein the AGP enzyme encoded by said polynucleotide molecule consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO. 5 and SEQ ID NO. 3.

6. The polynucleotide molecule, according to claim 5, wherein the nucleotide sequence encoding SEQ ID NO. 3 comprises nucleotides 87 through 1640 of the sequence shown in SEQ ID NO. 2 or a degenerate fragment thereof.

7. A method for increasing the seed weight of a plant, comprising incorporating the polynucleotide molecule of claim 1 into the genome of said plant and expressing the protein encoded by said polynucleotide molecule.

8. The method, according to claim 7, wherein said plant is *Zea mays*.

9. A plant seed comprising the polynucleotide molecule of claim 1 within the genome of said seed.

10. A plant expressing the polynucleotide molecule of claim 1.

11. The plant, according to claim 10, wherein said plant is *Zea mays*.

12. The plant, according to claim 10, wherein said plant is grown from the seed of claim 9.

13. A variant ADP-glucose pyrophosphorylase (AGP) protein, wherein said protein has at least one additional amino acid inserted within or close to the allosteric binding site of the wild-type AGP protein.

14. The variant AGP protein, according to claim 13, wherein said protein has at least one serine residue inserted between amino acids 494 and 495 of the wild type AGP protein sequence.

15. The variant AGP protein, according to claim 11, wherein said protein has the amino acid pair tyrosine:serine inserted between amino acids 494 and 495 of the wild-type AGP protein sequence.

16. The variant AGP protein, according to claim 11, wherein said protein has the amino acid pair serine:tyrosine inserted between amino acids 495 and 496 of the wild-type AGP protein sequence.

17. The variant AGP protein, according to claim 13, wherein said protein consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO. 5 and SEQ ID NO. 3.

18. The variant AGP protein, according to claim 13, wherein said protein is expressed in the endosperm of a plant during seed development.

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 96/14244

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N9/12 C12N15/54 A01H5/00 A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROC. NATL. ACAD. SCI. USA, vol. 93, no. 12, 11 June 1996, pages 5824-9, XP000652281 M.J. GIROUX ET AL.: "A single gene mutation that increases maize seed weight" see the whole document. ---	1-18
A	PLANT CELL, vol. 2, 1990, pages 581-8, XP000652283 M.R. BHAVE ET AL.: "Identification and molecular characterization of Shrunken-2 cDNA clones of maize" cited in the application see the abstract. -----	1

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 1997	20.06.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer Yeats, S

Form PCT ISA 210 (second sheet) (July 1992)